Mycalosides B−I, Eight New Spermostatic Steroid Oligoglycosides from the Sponge *Mycale laxissima*

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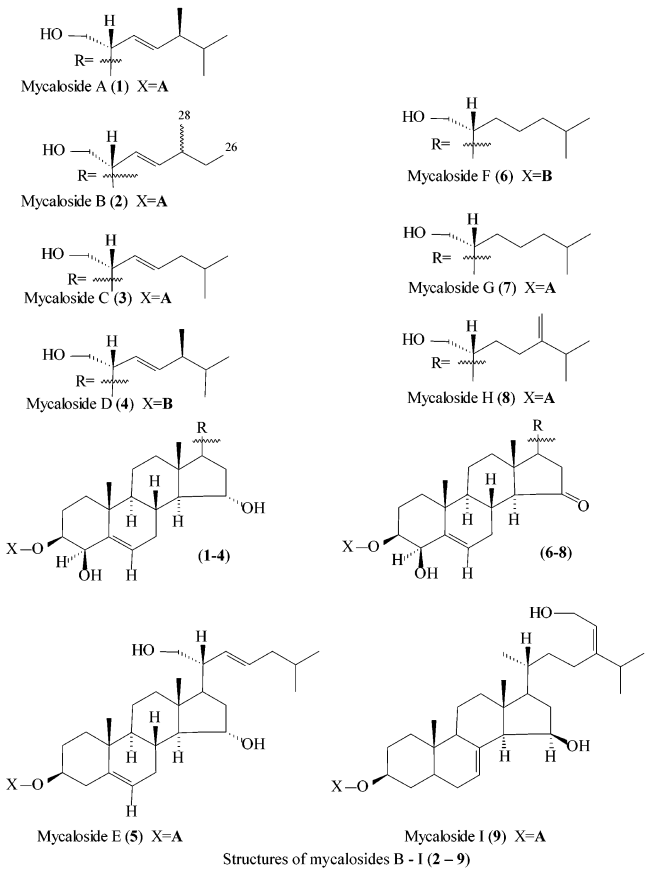
New steroidal oligoglycosides mycalosides B−I (**2**−**9**) have been isolated from the polar extract of the Carribean sponge *Mycale laxissima*, and their structures have been elucidated by 1D and 2D NMR (^{1}H, ^{13}C, DEPT, COSY-45, COSY-RCT, HSQC, HMBC, and NOESY spectra) and MALDI-TOF mass spectrometry. Mycalosides B (**2**) and C (**3**) were shown to be 27- and 28-nor derivatives of the previously known tetraoside mycaloside A (**1**). Mycaloside D (**4**) differs from **1** only in the presence of an additional acetyl group in the carbohydrate moiety. Mycaloside E (**5**) was structurally identified as a 28-nor-4-dehydroxy derivative of **1**. Mycalosides F−H (**6**−**8**), differing from each other by the structures of their side chains and nonacetylated (**7**, **8**) or acetylated (**6**) tetrasaccharide carbohydrate moieties, have new 5(6)-unsaturated 3β,4β,21-trihydroxy-15-keto-steroidal aglycons. Mycaloside I (**9**) is a tetraoside of a new 7,24(28)-diunsaturated 3β,15β,29-trihydroxystigmastane aglycon. It was established that the total fraction of the mycalosides as well as mycalosides A (**1**) and G (**7**) inhibit the fertilization of eggs by sperm of the sea urchin *Strongylocentrotus nudus* preincubated with these compounds.

Steroidal oligoglycosides have long been considered as typical secondary metabolites of higher plants until these compounds were found as predominant metabolites in some marine invertebrates, namely, starfish.[1−3] Later, related glycosides, mainly of the norlanostane series, were isolated from sponges. To the best of our knowledge, about two dozens sponge norlanostane glycosides have been isolated to date, including sarasinosides from *Asteropus* spp.[4−7] and *Melophlus isis*,[8] erylosides and formosides from *Erylus* spp.,[9−12] ulososides from *Ulosa* sp.,[13−15] and ectyoplasides and feroxosides from *Ectyoplasia ferox*.[16,17] In addition, a few steroid biosides were found from some sponge species.[18,19] Recently, we have reported the isolation of a new steroid oligoglycoside, mycaloside A (**1**), with unprecedented structural features in both the carbohydrate and aglycon moieties from the Caribbean sponge *Mycale laxissima*.[20] Further separation of the glycoside fraction from *M. laxxisima* has now led to the isolation of eight new steroid oligoglycosides, mycalosides B−I (**2**−**9**). In this paper we described the isolation and structural elucidation of these glycosides, which differ from mycaloside A in their aglycon or/and carbohydrate moieties.



Structures of mycalosides B - I (2 – 9)

Results and Discussion

The ethanolic extract of the sponge was partitioned between water and *n*-butanol, followed by column chromatography of the butanol-soluble portion on Teflone powder (Polychrome-1) and elution with H_2O and 50% EtOH. Further column chromatography of the 50% EtOH eluate on Silicagel L, with a solvent gradient system of increasing polarity from EtOAc to EtOH, gave the glycoside fraction. The separation of this fraction using HPLC on reversed-phase sorbents yielded mycalosides B−I (**2**−**9**) along with the previously described mycaloside A (**1**)[20] as white amorphous solids.

A preliminary inspection of the NMR spectra (Tables 1−3) suggested the glycosteroid nature of all of the isolated compounds. Spectra exhibited the signal characteristic of steroid aglycons with two singlets and two or three doublets of methyl groups in tetracyclic moieties and side chains, respectively. In the ^{13}C NMR spectra (DEPT), four CH signals for anomeric carbons in the range δ 99.3−104.4 showed that each of these glycosides was a tetraoside.

Analysis of NMR spectra allowed us to establish that the majority of the isolated glycosides had the same carbohy-

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Table 1. ^{1}H, ^{13}C NMR, and NOESY Data of the Carbohydrate Chains **A** and **B**

	carbohydrate chain **A**				carbohydrate chain **B**		
	δ_C DEPT	δ_H (*J*, Hz)	NOESY (H)		δ_C DEPT	δ_H (*J*, Hz)	NOESY (H)
		Glc (1→C-3)				6-OAcGlc (1→C-3)	
1	101.8 CH	4.90 d (7.9)	H3,4; H3,5-Glc	1	101.9 CH	4.88 d (7.9)	H3; H3,5-6-OAcGlc
2	76.3 CH	4.21 t (9.1)	H4-Glc	2	76.0 CH	4.23 t (8.2)	H4-6-OAcGlc
3	77.4 CH	4.48 t (9.2)	H1,2-Glc	3	77.2 CH	4.44 m	H1-6-OAcGlc H1-Ara
4	74.5 CH	4.72 t (9.6)	H2-Glc; H1-Gal 2	4	75.3 CH	4.46 m	H2,6-6-OAcGlc H1-Gal 2
5	77.6 CH	3.74 m	H1-Glc	5	74.3 CH	3.92 m	H1,6-6-OAcGlc
6	60.6 CH_2	4.45 m 4.73 m		6	63.3 CH_2	5.07 brd (3.3)	H4,5-6-OAcGlc H1-Gal 2
				Ac	170.4 C 20.6 CH_3	2.01 s	
		Ara (1→3Glc)				Ara (1→3Glc)	
1	99.3 CH	6.62 d (3.6)	H2-Ara; H3-Glc	1	99.3 CH	6.63 d (3.5)	H2-Ara; H3-6-OAcGlc
2	80.1 CH	4.74 m	H1-Ara; H1-Gal 1	2	80.4 CH	4.72 dd (3.4; 10.0)	H1-Ara; H1-Gal 1
3	69.1 CH	5.20 dd (3.6; 10.0)	H5-Ara	3	69.0 CH	5.16 dd (3.4; 10.0)	H4-Ara
4	70.7 CH	4.85 brd (3.3)	H5-Ara	4	70.6 CH	4.83 brd (3.3)	H3,5-Ara
5	64.4 CH_2	5.52 brd (12.0) 4,00 brd (12.0)	H4-Ara; H3-Ara	5	64.3 CH_2	5.45 brd (11.4) 3.99 brd (11.4)	H4-Ara
		Gal 1 (1→2Ara)				Gal 1 (1→2Ara)	
1	103.3 CH	5.77 d (3.8)	H2-Gal 1; H2-Ara	1	103.6 CH	5.73 d (3.5)	H2-Gal 1; H2-Ara
2	71.1 CH	4.61 dd (3.7; 10.0)	H1-Gal 1	2	71.0 CH	4.62 dd (3.4; 9.9)	
3	71.2 CH	4.56 dd (3.7; 9.9)	H5-Gal 1	3	71.2 CH	4.60 dd (3.0; 10.0)	
4	71.0 CH	4.37 brd (3.5)	H5-Gal 1	4	71.1 CH	4.41 brs	H5-Gal 1
5	73.2 CH	4.81 brt (5.5)	H3,4-Gal 1	5	73.3 CH	4.84 m	H4-Gal 1
6	62.9 CH_2	4.27 dd (4.2; 11.1) 4.40m		6	63.0 CH_2	4.29 dd (4.2; 11.2) 4.44 m	
		Gal 2 (1→4 Glc)				Gal 2 (1→4 6-OAcGlc)	
1	104.2 CH	5.32 d (7.9)	H3,5-Gal 2; H4-Glc	1	104.7 CH	4.90 d (7.7)	H3,5-Gal 2; H4,6-6-OAcGlc
2	72.7 CH	4.42 t (8.7)		2	72.5 CH	4.41 dd (7.7; 9.4)	
3	74.8 CH	4.11 dd (3.3; 9.5)	H1,5-Gal 2; H4-Gal 2	3	74.7 CH	4.07 dd (3.4; 9.4)	H4-Gal 2; H4-6-OAcGlc
4	69.0 CH	4.48 brs	H3,5-Gal 2	4	69.0 CH	4.52 brs (3.3)	H3,5-Gal 2
5	76.2 CH	3.95 brt (6.1)	H1,3,4-Gal 2	5	76.5 CH	3.94 brt (6.9)	H1,4-Gal 2
6	61.5 CH_2	4.33 dd (6.0; 10.6) 4.39 m		6	61.4 CH_2	4.33 dd (5.8; 10.7) 4.45 m	

Table 2. ^{13}C NMR Spectra of Aglycon Moieties of Mycalosides B I (δ, dept)

C	B (**2**)	C (**3**)	D (**4**)	E (**5**)	F (**6**)	G (**7**)	H (**8**)	I (**9**)
1	37.5 CH_2	37.5 CH_2	37.6 CH_2	37.4 CH_2	37.4 CH_2	37.4 CH_2	37.5 CH_2	37.2 CH_2
2	23.8 CH_2	23.8 CH_2	23.8 CH_2	30.0 CH_2	23.7 CH_2	23.8 CH_2	23.8 CH_2	29.8 CH_2
3	80.1 CH	80.1 CH	80.7 CH	78.7 CH	80.3 CH	80.1 CH	80.1 CH	77.9 CH
4	74.3 CH	74.5 CH	74.5 CH	39.1 CH	74.5 CH	74.5 CH	74.3 CH	34.5 CH_2
5	141.8 C	141.8 C	141.9 C	140.0 C	142.3 C	142.1 C	142.2 C	39.9 CH
6	128.6 CH	128.7 CH	128.6 CH	122.4 CH	127.6 CH	127.7 CH	127.7 CH	29.8 CH_2
7	32.9 CH_2	32.9 CH_2	32.9 CH_2	32.7 CH_2	31.7 CH_2	31.6 CH_2	31.6 CH_2	118.5 CH
8	32.1 CH	32.0 CH	32.0 CH	32.0 CH	28.3 CH	28.2 CH	28.2 CH_2	136.7 C
9	50.8 CH	50.7 CH	50.8 CH	50.4 CH	50.1 CH	50.0 CH	50.0 CH	49.6 CH
10	36.5 C	36.5 C	36.5 C	36.7 C	36.2 C	36.2 C	36.2 C	34.4 C
11	20.5 CH_2	20.5 CH_2	20.5 CH_2	21.0 CH_2	20.4 CH_2	20.3 CH_2	20.4 CH_2	21.6 CH_2
12	39.6 CH_2	39.6 CH_2	39.6 CH_2	39.6 CH_2	38.9 CH_2	38.8 CH_2	38.9 CH_2	40.0 CH_2
13	43.2 C	43.2 C	43.2 C	43.2 C	42.1 C	42.0 C	42.1 C	44.1 C
14	63.4 CH	63.4 CH	63.3 CH	63.2 CH	66.5 CH	66.4 CH	66.5 CH	62.5 CH
15	73.3 CH	73.2 CH	73.4 CH	73.4 CH	215.3 C	215.3 C	215.2 C	69.6 CH
16	40.8 CH_2	40.8 CH_2	41.0 CH_2	40.8 CH_2	41.0 CH_2	41.0 CH_2	41.0 CH_2	40.6 CH_2
17	48.5 CH	48.6 CH	48.7 CH	48.6 CH	46.8 CH	46.7 CH	46.6 CH	53.5 CH
18	13.8 CH_3	13.8 CH_3	13.8 CH_3	13.8 CH_3	12.9 CH_3	12.9 CH_3	12.9 CH_3	13.2 CH_3
19	21.0 CH_3	21.0 CH_3	21.1 CH_3	19.3 CH_3	20.8 CH_3	20.8 CH_3	20.8 CH_3	13.0 CH_3
20	48.2 CH	48.3 CH	48.3 CH	48.4 CH	42.2 CH	42.2 CH	42.1 CH	36.6 CH
21	64.8 CH_2	64.8 CH_2	64.8 CH_2	64.7 CH_2	62.1 CH_2	61.9 CH_2	61.9 CH_2	18.7 CH_3
22	131.5 CH	134.3 CH	132.3 CH	134.3 CH	30.1 CH_2	30.0 CH_2	28.7 CH_2	36.3 CH_2
23	136.9 CH	129.7 CH	135.2 CH	129.7 CH	24.5 CH_2	24.4 CH_2	31.5 CH_2	26.5 CH_2
24	38.8 CH	42.2 CH_2	43.5 CH_2	42.2 CH_2	39.6 CH_2	39.6 CH_2	156.5 C	147.1 C
25	30.0 CH_2	28.5 CH	33.4 CH	28.6 CH	28.1 CH	28.1 CH	34.0 CH	34.8 CH
26	11.9 CH_3	22.3 CH_3	19.7 CH_3	22.4 CH_3	22.7 CH_3	22.5 CH_3	21.9 CH_3	22.1 CH_3
27		22.2 CH_3	20.2 CH_3	22.2 CH_3	22.6 CH_3	22.7 CH_3	21.8 CH_3	22.0 CH_3
28	20.6 CH_3		18.1 CH_3				106.6 CH_2	123.7 CH
29								58.8 CH_2

drate moiety, with D-glucose, D-arabinose, and two D-galactose residues, as in mycaloside A (**1**). This carbohydrate fragment (**A**) was found to be in glycosides **2**, **3**, **5**, and **7**−**9**. The other mycalosides (**4**, **6**) contained carbohy-

Table 3. ^{1}H NMR Spectral Data δ (J, Hz) of Aglycon Moieties of Mycalosides B−I (**2**−**9**)

H	B (**2**)	C (**3**)	D (**4**)	E (**5**)	H	F (**6**)	G (**7**)	H (**8**)	I (**9**)
1	1.77 m (e)	1.77 m (e)	1.72 m (e)	1.73 m (e)	1	1.71 m (e)	1.73 m (e)	1.72 m (e)	1.66 m (e)
	1.02 m (a)	1.03 m (a)	1.03 m (a)	0.98 m (a)		1.01 m (a)	1.00 m (a)	0.98 m (a)	0.90 m (a)
2	1.79 m (e)	1.80 m (e)	1.78 m (e)	1.32 m (e)	2	1.82 m (e)	1.79 m (e)	1.78 m (e)	1.91 m (e)
	2.32 m (a)	2.31 m (a)	2.29 m (a)	1.63 m (a)		2.27 m (a)	2.29 m (a)	2.30 m (a)	1.46 m (a)
3	3.85 m	3.85 dt (3.7; 11.9)	3.84 m	3.72 m	3	3.83 dt (3.5; 11.3)	3.83 dt (3.9; 12.0)	3.83 dt (3.8; 11.6)	3.75 m
4	4.58 brd (3.7)	4.58 brd (3.5)	4.57 brd (3.4)	2.54 m (e)	4	4.54 brd (3.5)	4.65 brd (3.1)	4.55 brd (3.1)	1.84 m (e)
				2.33 m (a)					1.25 m (a)
5					5				1.12 m
6	5.74 dd (2.0; 5.3)	5.73 dd (2.5; 5.3)	5.72 dd (2.5; 5.3)	5.36 m	6	5.64 dd (2.4; 5.2)	5.63 dd (2.5; 5.1)	5.63 dd (2.5; 5.1)	1.68 m
7	2.27 m	2.70 dt (5.3; 19.0)	2.26 m	2.59 m					1.62 m
	2.71 dt (5.6; 19.0)	2.27 m	2.77 dt (5.5; 19.0)	2.18 m	7	3.18 dt (5.5; 18.9)	3.19 m	3.19 dt (5.1; 18.4)	5.80 m
8	1.89 m	1.88 m	1.87 m	1.72 m		1.75 m	1.76 m	1.75 m	
9	1.01 m	1.01 m	1.01 m	1.03 m	8	1.95 m	1.99 m	1.96 td (5.4; 10.6)	
10					9	0.93 m	0.93 m	0.92 m	1.57 m
11	1.46 m	1.47 m	1.41 m	1.47 m	10				
12	2.02 m (e)	2.01 m (e)	1.40 m (a)	2.03 m (e)	11	1.43 m	1.44 m	1.43 m	1.47 m
	1.40 m (a)	1.39 m (a)	2.01 m (e)	1.39 m (a)	12	1.55 m (a)	2.23 m (e)	2.22 m (e)	1.95 m (e)
13						2.22 m (e)	1.53 m (a)	1.54 brt (12.6) (a)	1.28 m (a)
14	1.45 m	1.44 m	1.44 m	1.43 m	13				
15	4.22 m	4.21 m	4.21 m	4.20 td (3.3; 9.0)	14	1.83 d (11.1)	1.84 d (10.8)	1.85 d (10.7)	2.19 m
16	2.18 m	2.18 m	2.18 m	2.17 m	15				4.46 m
	2.04 m	2.04 m	2.05 m	2.08 m	16	2.02 m	2.02 m	2.56 dd (9.0; 18.2)	2.02 m
17	2.04 m	2.02 m	2.06 m	2.06 m		2.55 dd (9.5; 18.0)	2.55 dd (8.7; 18.2)	2.02 m	2.17 m
18	0.83 s	0.82 s	0.82 s	0.82 s	17	2.22 m	2.21 m	2.23 m	1.56 m
19	1.40 s	1.40 s	1.42 s	0.88 s	18	0.79 s	0.80 s	0.80 s	0.63 s
20	2.37 m	2.40 m	2.36 m	2.42 m	19	1.30 s	1.32 s	1.32 s	0.66 s
21	3.74 m	4.03 dd (4.1; 10.4)	3.77 dd (7.8; 10.7)	4.02 m	20	1.71 m	1.71 m	1.77 m	1.38 m
	4.01 m	3.75 m	4.00 m	3.75 dd (7.2; 10.2)	21	3.98 m	4.00 m	4.03 m	0.99 d (6.5)
22	5.50 dd (9.0; 15.3)	5.53 m	5.48 dd (8.8; 15.3)	5.53 m		3.91 dd (4.5; 10.5)	3.91 dd (4.9; 10.7)	3.92 m	
23	5.40 dd (7.7; 15.3)	5.54 m	5.45 dd (7.7; 15.3)	5.54 m	22	1.54 m	1.54 m	1.77 m	1.56 m
24	2.02 m	1.91 m	1.94 m	1.92 m					1.25 m
		1.85 m			23	1.40 m	1.54 m	2.20 m	2.21 m
25	1.28 m	1.59 m	1.49 m	1.62 m		1.53 m	1.40 m	2.34 m	2.01 m
26	0.86 t (7.4)	0.87 d (6.6)	0.86 d (6.8)	0.88 d (6.6)	24	1.16 m	1.21 m		
27		0.88 d (6.6)	0.86 d (6.8)	0.87 d (6.6)	25	1.50 m	1.53 m	2.28 m	2.28 sept (7.0)
28	0.97 d (6.7)		0.96 d (6.9)		26	0.87 d (6.6)	0.87 d (6.6)	1.04 d (6.8)	1.05 d (7.0)
					27	0.87 d (6.6)	0.87 d (6.6)	1.05 d (6.8)	1.05 d (7.0)
					28			4.86 brs	5.72 t (6.5)
					29				4.56 d (6.5)

drate moiety **B**. NMR data for the carbohydrate chains in glycosides **2** and **4** are given in Table 1. In the other mycalosides, the corresponding parts of the spectra are very similar to those of either **2** or **4**.The structures of fragments **A** and **B** in these new mycalosides were established by COSY-45, COSY-RCT, HSQC, HMBC, and NOESY experiments. Sizes of monosaccharide rings and configurations of glycoside bonds followed from spin-decoupling constants and NOESY data (Table 1). The presence of an additional acetoxy group (δ 2.01, s, 170.4, C, 20.6, CH_3) attached to C-6 in the glucosyl residue of **B** influences the chemical shifts of C-6 and C-5 of this monosaccharide in accordance with the known α- and β-effects of acetylation (Table 1). Inasmuch as chemical shifts of H-3 and H-4 in the β-D-glucopyranose residue of **B** are similar to each other, the determination of positions of β-D-galactopyranose and β-D-arabinopyranose at C-3 or C-4 of the glucose residue was not reliable by HMBC and NOESY methods when we used these signals. The attachment of the galactopyranosyl residue to C4 of glucose has been established by observation of NOE between H6 of the glucopyranosyl residue (δ 5.07) and H1 of the galactopyranosyl residue (δ 4.90) (Table 1).

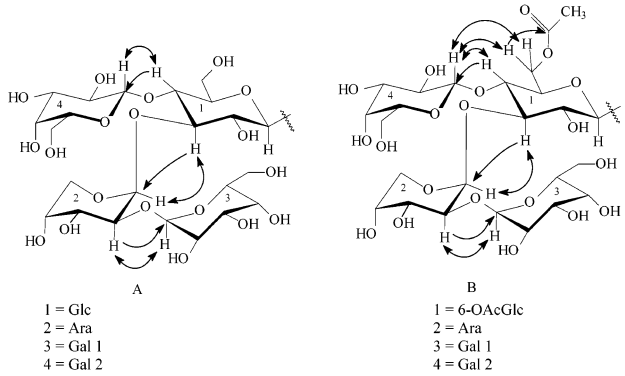

Figure 1. Structures of the carbohydrate chains (**A** and **B**) of the mycalosides and some key HMBC (→) and NOESY (↔) correlations.

Table 4. Selected HMBC Correlations of Aglycon Moieties of Mycalosides B−I (**2**−**9**) (H/C)

	B (**2**)	C (**3**)	D (**4**)	E (**5**)
H1-Glc				C-3
H-3	C1-Glc	C1-Glc	C1-6-OAcGlc	
H-4	C-6	C-6	C-6	
H-6	C-8	C-8	C-8	
H_3-18	C-12, 13, 14, 17	C-12, 13, 14, 17	C-12, 13, 14, 17	C-12, 13, 14, 17
H_3-19	1, 5, 9, 10	1, 5, 9, 10	C-1, 5, 9, 10	C-5, 9, 10
H_3-21	C-22	C-22	C-22	C-22
H-25		C-23		C-23
H_3-26	C-24, 25	C-24, 25, 27	C-24, 25, 27	C-24, 25, 27
H_3-27		C-24, 25, 26	C-24, 25, 26	C-24, 25, 26
H_3-28	C-23, 24, 25		C-23, 24, 25	

	F (**6**)	G (**7**)	H (**8**)	I (**9**)
H1-6-OAcGlc	C-3			
H1-Glc		C-3		
H-3			C1-Glc	C1-Glc
H-4	C-10		C-6	
H-6			C-8	
H-15				C-8
H_3-18	C-12, 13, 14, 17	C-12, 13, 14, 17	C-12, 13, 14, 17	C-12, 13, 14, 17
H_3-19	C-5, 9, 10	C-1, 5, 9, 10	C-1, 5, 9, 10	C-1, 5, 9, 10
H_3-21	C-22		C-22	C-17, 20
H_3-26	C-24, 25, 27	C-24, 25, 27	C-24, 25, 27	C-24, 25, 27
H_3-27	C-24, 25, 26	C-24, 25, 26	C-24, 25, 26	C-24, 25, 26
H-28			C-23, 25	C-23
H_3-29				C-24

Mycaloside B (**2**) analyzed for $C_{50}H_{82}O_{23}$ by combined HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses. ^1H and ^{13}C NMR spectra of **2** (Tables 1−3) indicated the presence of a carbohydrate chain of the **A** type and a steroid aglycon oxidized in positions 3β, 4β, 15α, and 21 with an unsaturated side chain having eight carbon atoms. The cross-peak H-3 (δ 3.85)/C1-Glc (δ 101.8) in the HMBC spectrum (Table 4) showed that the carbohydrate chain is attached to C-3 of the aglycon. The structure of the tetracyclic portion of the aglycon was confirmed by 2D NMR experiments in the same manner as was carried out previously for mycaloside A.[20] The presence of a hydroxyl group at C-21 (δ H_2-21, 3.74, m, and 4.01, m) and a 22*E* double bond (δ 131.5, CH, and 136.9, CH, $J_{22,23}$ = 15.3 Hz) and the absence of a signal for one secondary methyl group in the ^1H NMR spectrum when compared with that of **1** suggested that **2** is probably the 27-nor analogue of **1**. The structure of the side chain was confirmed by COSY-45, COSY-RCT, and especially HMBC data. In fact, HMBC correlations between a triplet signal of H_3-26 (δ 0.86) and a doublet signal of C-24 (δ 38.8), as well as between a doublet signal of H_3-28 (δ 0.97) and the same signal of C-24 and the signal of C-25 (δ 30.0), showed the attachment of ethyl and methyl groups to C-24. On the basis of all of the above data (see Tables 1−3), the structure of mycaloside B (**2**) was established as 27-nor-mycaloside A. A 20*R*-configuration in mycaloside B followed from NOESY correlations (see Experimental Section) between H-20 (δ 2.37, m) and H_3-18 (δ 0.83) and between H_2-21 (δ 3.74, m and 4.01, m) and H-12e (δ 2.02, m). The configuration of C-24 was not determined.

Mycaloside C (**3**) also analyzed for $C_{50}H_{82}O_{23}$ by combined HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses. The comparison of NMR data of mycaloside C and mycalosides A and B (**1**, **2**) (Tables 1−3) suggested that **3** is an isomer of **2**. The spectra for the tetracyclic portion of the aglycon and carbohydrate chain of **3** coincided with those of **1** and **2**. This was confirmed by 2D NMR experiments. The 21-hydroxy-22-dehydrocholestane structure of the side chain was established using COSY-45 and HMBC correlations (Table 4). For example, HMBC correlations between H_2-21 (δ 4.03 and 3.75) and C-22 (δ 134.3) as well as between H_3-26, H_3-27 (δ 0.87 and 0.88), and C-24 (δ 42.2) confirmed this structure. Because the chemical shifts of H-22 and H-23 (δ 5.53 and 5.54) were overlapped, it was impossible to use the value of H-22, H-23 coupling constants for the determination of the 22(23) double-bond configuration. However, the downfield shift of the C-24 signal to δ 42.2 in comparison with the usual for *cis*-double bonds ($\delta_{\text{allylic carbon}} \approx 36-37$) indicated this double bond has a *trans*-configuration in **3**.[21] Therefore, **3** is 28-nor-mycaloside A.

The molecular formula of mycaloside D (**4**) was determined as $C_{53}H_{86}O_{24}$ by the pseudomolecular ion at *m*/*z* 1129.5462 in HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). The structure of the aglycon was established by 2D NMR experiments (see Table 4 and Experimental Section) as (24*S*)-24-methyl-3β,4β,15α,21-tetrahydroxycholesta-5,22-diene, the same as in mycaloside A (**1**).[20] The structure of the carbohydrate chain was determined as the **B** type, as described above (Table 1). On this basis, it was established that **4** was the monoacetate of mycaloside A (**1**), having an acetoxy group at C6 of the glucose residue.

Mycaloside E (**5**) analyzed for $C_{50}H_{82}O_{22}$ by combined HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). NMR studies suggested that **5** was a dehydroxy analogue of mycaloside C. In fact, COSY-45, COSY-RCT, and HSQC spectra along with HMBC correlations (Table 4) confirmed the presence of a hydroxyl group at C-15 and not at C-4. An α-configuration of the hydroxyl group at C-15 was established by coupling constants (H-15, δ 4.20, td, J = 9.0, 3.3 Hz) and the NOESY correlation between H-15 and H-8 (δ 1.72).

NMR data demonstrated the structure of the side chain to be the same as that of **3** (Tables 2 and 3), showing the very close similarity of the corresponding parts of the ^1H and ^{13}C NMR spectra in both compounds. The cross-peak H1-Glc (δ 4.78)/C-3 (δ 78.7) in the HMBC spectrum established that the carbohydrate chain is attached to C-3 of the aglycon. On this basis it was concluded that **5** is the 4-dehydroxy derivative of mycaloside C (**3**).

The molecular formula of mycaloside F (**6**) was determined as $C_{52}H_{84}O_{24}$ by the pseudomolecular ion at *m*/*z* 1115.5228 in HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). The carbohydrate moiety was identified as the **B** type. The connectivity sequences of protons in rings A and B ($-CH_2-CH_2-CH(-O-)-CH(-OH)-$ and $=CH-CH_2-CH<$) were revealed by the COSY-45 NMR spectrum. This, together with data of HMBC experiments (Table 4), especially with correlations between H-4 (δ 4.54) and C-10 (δ 36.2) and between H_3-19 (δ 1.30) and C-5 (δ 142.3), gave the structures of rings A and B in **6**.

The chemical shift of the carbonyl carbon signal (δ 215.3) was characteristic of a ketone group in a five-membered ring. The multiplicities and chemical shifts of H-14 (a doublet at δ 1.83) and C-14 (CH, δ 66.5) indicated that this ketone group was located at C-15. A *trans*-C/D ring juncture followed from observing $J_{8,14}$ = 11.1 Hz. The β-configurations of the oxygenic substituents at C-3 and C-4 were determined from the coupling constants of H-3 (a doublet of triplets at δ 3.83, J = 11.3, 3.5 Hz) and H-4 (a broad doublet at δ 4.54, J = 3.5 Hz) and data of the NOESY spectrum (see Experimental Section).

It was impossible to determine whether protons from H-17 or H-12e caused the cross-peak with H_2-21 in the NOESY spectrum because of the overlapping chemical shifts of these atoms (multiplets at δ 2.22). However, the presence of cross-peaks H-16 (δ 2.55)/H-22 (δ 1.54) and H-20 (δ 1.71)/H_3-18 (δ 0.79) in this spectrum confirmed the 20*R*-configuration in **6**. The attachment of the carbohydrate chain to C-3 was established by the correlation of H1-6-OAcGlc (δ 4.88, d)/C-3 (δ 80.3) in the HMBC spectrum and the downfield chemical shift of C-3 (δ 80.3). Therefore, mycaloside F (**6**) is an acetylated tetraoside of a new aglycon, namely of (20*R*)-3β,4β,21-trihydroxycholest-5-en-15-one.

Mycaloside G (**7**) analyzed for $C_{50}H_{82}O_{23}$ by combined HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). These data and NMR spectra suggested that **7** was a deacetylated derivative of **6**. This was confirmed by COSY-45, HMBC, and NOESY spectra. In fact, the aglycone of **7** was identical to that of **6**. The carbohydrate moiety was established to be of **A** type (Table 1). Its attachment to C-3 was shown by the chemical shift of C-3 (δ 80.1) and the H1-Glc (δ 4.90)/C-3 correlation in the HMBC spectrum (Table 4).

Mycaloside H (**8**) analyzed for $C_{51}H_{82}O_{23}$ by combined HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). The carbohydrate chain of the **A** type was identified. The structures of rings A and B were established in the same manner as for mycaloside B (**2**). The structures of rings C and D were established in the same manner as for mycaloside F (**6**). The *trans*-C/D ring juncture followed from observing $J_{8,14}$ = 10.7 Hz.

The 21-hydroxy-24-methylene side chain was identified by NOESY (see Experimental Section) and HMBC experiments (Table 4). Protons at C-21 (multiplets at δ 4.03 and 3.92) gave cross-peaks with C-22 (δ 28.7) in the HMBC spectrum and with H-12e (δ 2.22) in the NOESY spectrum, while protons at C-28 (δ 4.86) gave cross-peaks with C-23 (δ 31.5) and C-25 (δ 34.0 ppm). Protons of H_3-26 and H_3-27 (δ 1.04 and 1.05) gave cross-peaks with C-24 (δ 156.5) in the HMBC spectrum. Attachment of the carbohydrate moiety to C-3 was shown by the chemical shift of C-3 (δ 80.1) and the H-3 (δ 3.83)/C1-Glc (δ 102.0) correlation in the HMBC spectrum. On the basis of these data, the structure of mycaloside H (**8**) was established as the corresponding tetraoside of the previously unknown aglycon, (20*R*)-3β,4β,21-trihydroxyergosta-5,24(28)-dien-15-one.

Finally, the molecular formula of mycaloside I (**9**) was determined as $C_{52}H_{86}O_{22}$ by the pseudomolecular ion at *m*/*z* 1085.5481 in HR MALDI TOF MS (positive mode) and ^{13}C NMR analyses (Tables 1 and 2). The carbohydrate chain of the **A** type was identified by analyses of NMR spectra. Chemical shifts at δ 118.5 (CH) and 136.7 (C) in DEPT,[22] together with HMBC correlations of angular methyl groups (Table 4), showed that **9** contains the 7(8) double bond in the tetracyclic part of the aglycon. The COSY-45 and HSQC spectra revealed the connectivity sequence of the protons in rings A and B ($-CH_2-CH_2-CH(-O-)CH_2-CH(-C)-CH_2-CH=$). A 3$\beta$-configuration followed from NOESY correlations H-3 (δ 3.75)/H-5 (δ 1.12) and H-3 (δ 3.75)/H-1a (δ 0.90). The presence of a hydroxyl group at C-15 was established by the HMBC correlation H-15 (δ 4.46)/C-8 (δ 136.7) and the chemical shift of C-14 (δ 62.5). Its β-orientation was established by the H-15/H-7 (δ 5.8) correlation in the NOESY spectrum.

The 29-hydroxy-24-ene fragment in the side chain was identified by COSY-45 correlations as well as by correlations H_2-29 (δ 4.56)/C-24 (δ 147.1) and H-28 (δ 5.72)/C-23 (δ 26.5) in the HMBC spectrum. The *E*-configuration of the 24(28) double bond was determined on the basis of the chemical shift value and multiplicity of the C-25 methine proton. It was previously established that 24-hydroxyethyl-24(28)-ene structures with *E*-configuration show the signal for this proton as a septet at δ 2.32 (in the *Z* isomer it would be expected to give a signal shifted downfield to δ 3.0).[23] The septet of H-25 was at δ 2.28 in the ^1H NMR spectrum of **9** (Table 3). This configuration was also supported by NOESY correlations (see Experimental Section). The presence of the cross-peak between H-3 (δ 3.75) and C1-Glc (δ 102.4) in the HMBC spectrum confirmed the attachment of a carbohydrate moiety to C-3. On the basis of these data it was established that **9** is a tetraoside of a new steroidal aglycon, (20*R*)-3β,15β,29-trihydroxystigmasta-7,24(28)*E*-diene.

Thus, the structures of these eight new steroidal oligosides (**2**−**9**) were established. The glycosides (**2**−**8**) are derivatives either of the same aglycon that is in the previously described mycaloside A[20] or of new polyhydroxylated Δ^5-sterols. In contrast with other glycosides of this series, not only does mycaloside I (**9**) have a hydroxyl group at C-29 instead of C-21, but it is also an oligoglycoside of a new polyoxygenated Δ^7-steroidal aglycon.

It is of special interest that mycalosides F−H (**6**−**8**), which contain a ketone group at position 15, also have the *trans*-junction of rings C and D. It is known that the corresponding hydrindanone systems, when fused into more complicated structures such as steroids, frequently but not always have a more stable *cis*-C/D ring junction than the *trans*-junction.[24] Seven marine steroids possesing *cis*-C/D-ring junctions, namely, contignasterol from the sponge *Petrosia contignata*,[25] xestobergsterols A−C from the sponge *Xestospongia berquistia*[26] and *Ircinia* sp.,[27] haliclonostanone sulfate from the sponge *Haliclona* sp.,[28] clathriol from the sponge *Clathria lissosclera*,[29] and 14β-tamosterone sulfate from a new genus of Oceanapiidae,[30] are also 15-keto derivatives. However, tamosterone sulfate from the latter sponge, which also has the 15-ketone function, contains the *trans*-C/D ring junction and is quite stable, similar to **6**−**8**.

The total oligoglycoside fraction, as well as individual mycalosides A (**1**) and G (**7**), did not influence nonfertilized eggs and developing embryo up to the 8-blastomere stage

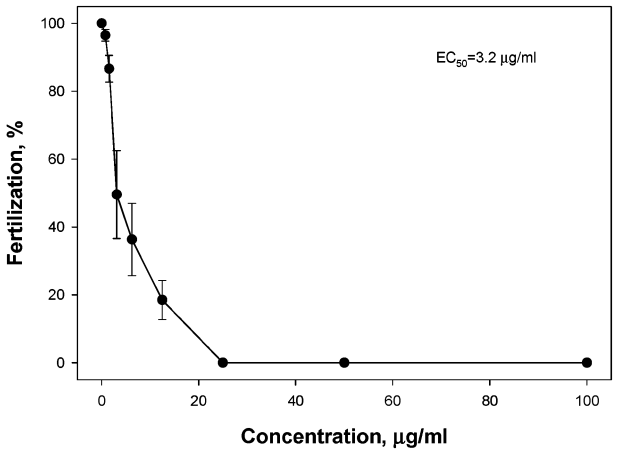

Figure 2. Effect of mycaloside A (**1**) on sea urchin sperm. Values are mean ± se, $n = 6$.

Table 5. Sterol Composition of the Sponge *Mycale laxissima*

sterol	RRT[a]	%[b]
27-nor-24-methylcholesta-5,22*E*-dien-3β-ol	0.89	4.4
cholesta-5,22*E*-dien-3β-ol	0.92	8.0
cholest-5-en-3β-ol (cholesterol)	1	23.2
5α-cholestan-3β-ol	1.02	1.7
24-methylcholesta-5,22*E*-dien-3β-ol	1.10	22.8
24-methylcholesta-5,24(28)-dien-3β-ol	1.24	13.1
24-methylcholest-5-en-3β-ol	1.25	2.1
24-ethylcholesta-5,22*E*-dien-3β-ol	1.34	8.4
24-ethylcholest-5-en-3β-ol	1.51	16.3

[a] The relative retention time of the sterol acetates under GC condition (RRT of cholesterol acetate = 1.0). [b] % of total sterol fraction.

in the concentration range 0−100 μg/mL. Only a weak delay of egg development was observed at a concentration of about 100 μg/mL. However, these compounds were effective as spermostatics when preincubated for 15 min with sea urchin sperm. Individual glycosides demonstrated the same levels of activities with an EC_{50} of 3.2 μg/mL, while the total fraction generated a less toxic effect (EC_{50} is 7.4 μg/mL) that was expressed as a percentage of sea urchin egg fertilization inhibition after artificial insemination. A typical dose−response plot is illustrated in Figure 2, which shows the effect of mycaloside A (**1**) upon sea urchin sperm and subsequent egg fertilization.

We suggest that the mycalosides are biosynthesized from free sterols of the sponge. In fact, our study on the composition of the free sterol fraction isolated from *M. laxissima* (Table 5) has shown a similarity between the structures of sterols and the corresponding mycalosides. For instance, cholesterol and 24-methylcholesta-5,22*E*-dien-3β-ol are the predominant constituents of the free sterol fraction. Side chains having the same structures as in these sterols, but additionally oxidized in position 21, were found in mycalosides F (**6**) and G (**7**) (the side chain of cholesterol) and mycalosides A (**1**) and D (**4**) (the side chain of 24-methylcholesta-5,22*E*-dien-3β-ol). 24-Methylenecholesterol and 24-ethylcholest-5-en-3β-ol were identified as other abundant free sterols. Mycalosides H (**8**) and I (**9**) contain similar side chains, but are additionally oxidized in positions 21 and 29, respectively. Finally, the side chain of mycaloside B (**2**) is similar to that of 27-nor-24-methylcholesta-5,22*E*-dien-3β-ol, and the side chains of mycalosides C (**3**) and E (**5**) resemble that of cholesta-5,22*E*-dien-3β-ol. Both of these sterols are conspicuous constituents of the free sterol fraction.

Experimental Section

General Experimental Procedures. The melting points were obtained with a Leica Galem II hot-stage apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in C_5D_5N with 5% of CD_3OD on a Bruker DPX-500 spectrometer operating at 500 and 125.8 MHz, respectively, using TMS as an internal standard. HRMALDI-TOF mass spectra were obtained on a Bruker Biflex instrument with a UV-nitrogen laser (337 nm). GLC-MS analyses were done on a Hewlett-Packard HP6890 GS system, using a HP-5MS capillary column (30.0 m × 250 μm × 0.25 μm) at 270 °C. The ionizing voltage was 70 eV. GLC analyses were performed on a Sigma 2000 Perkin-Elmer chromatograph using a capillary column (50 m × 0.2 mm) with CBP-5 at 290 °C. Helium was used as the carrier gas. Preparative HPLC was carried out on a Dupont-8800 chromatograph, using Silasoprb-ODS (10 μm, 9.6 × 200 mm), Diasphere-110-C18 (5 μm, 4 × 250 mm), and YMC-Pack ODS-A (5 μm, 10 × 250 mm) columns with an RIDK-22 refractometric detector. TLC was performed on precoated Si gel L (Chemapol, the former Czechoslovakia) glass plates (6 × 12 cm) in chloroform−EtOH−H_2O, 100:100:17, and detected by spraying with sulfuric acid (100 °C, 5 min).

Animal Material. The sponge was collected at a depth of 15 m using scuba near San-Felipe Island, Cuba, and identified as *Mycale laxissima* (Demospongiae, order Axinellida, family Mycaliidae) by V. B. Krasokhin. The voucher sample is under storage in the Pacific Institute of Bioorganic Chemistry, Vladivostok (PIBC 1-26). The sponge was cut and lyophilized immediately after collection.

Extraction and Isolation. The lyophilized specimens (0.3 kg) were macerated and sequently extracted with EtOAc (4 × 1 L) and EtOH (4 × 1 L). The EtOH extract concentrated in vacuo was partitioned between n-BuOH and H_2O. The n-BuOH-soluble portion (7.0 g) was separated by low-pressure reversed-phase column chromatography (the column 20 × 8 cm) on Teflon powder Polychrome-1 (Biolar, Latvia) in H_2O and 50% EtOH. After elution of inorganic salts and high-polar organic compounds by H_2O, 50% EtOH was used to obtain the fraction of amphiphilic compounds, including the mycalosides. The fraction obtained after evaporation in vacuo was subjected to Si gel flash column chromatography (6 × 9 cm) and eluted with a solvent gradient system of increasing polarity from EtOAc to EtOH. Fractions eluted with EtOAc−EtOH, 2:1, were combined, concentrated in vacuo (the residue 1.8 g), and separated by HPLC on a Silasorb ODS column with MeOH−H_2O (68:32) to obtain 10 subfractions.

Subfraction II (72 mg) was further purified and separated by HPLC on a Diasphere-110-C18 column in the same system and was then repeatedly chromatographed on a YMC-Pack ODS-A column in the solvent system MeOH−H_2O−$CHCl_3$ (62:38:4) to give mycalosides B (**2**) (9 mg) and C (**3**) (8 mg). Subfraction III (75 mg) was further purified and separated by HPLC on a Diasphere-110-C18 column with MeOH−H_2O−$CHCl_3$ (65:32:5) and eluted repeatedly with MeOH−H_2O−$CHCl_3$ (57:43:5) to give mycaloside E (**5**) (4.5 mg). Subfraction IV (145 mg) was subjected to HPLC on a Diasphere-110-C18 column with MeOH−H_2O−$CHCl_3$ (57:43:5) to give mycalosides A (**1**) (45.0 mg), F (**6**) (7 mg), and H (**7**) (10 mg). Mycalosides D (**4**) (16 mg) and G (**8**) (6 mg) were obtained from the subfraction V (110 mg) by HPLC on a Diasphere-110-C18 column with the solvent system MeOH−H_2O−$CHCl_3$ (65:32:5). Sufraction X (65 mg) was separated by HPLC on a Diasphere-110-C18 column with MeOH−H_2O−$CHCl_3$ (70:30:1) as an eluent system to give mycaloside I (**9**) (9 mg).

Mycaloside B (2): colorless solid, mp 218−222 °C; $[\alpha]^{25}_D$ −27.8° (*c* 0.8, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 3/1a,1-Glc, 4/6,1-Glc, 6/7,4, 7/6, 8/15,18, 11/1e, 12/21, 15/8,16,18, 16/15, 18/15,20,8, 20/18, 21/12e; HR MALDI MS (positive ions) *m*/*z* 1073.5077 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{23}Na$, 1073.5146), 1089.45 $[M + K]^+$.

Mycaloside C (3): colorless solid, mp 226−229 °C; $[\alpha]^{25}_D$ −28.2° (*c* 0.6, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 2e/19, 3/4,1a,1-Glc, 4/3,6,1-Glc, 6/7,4, 7/6, 8/15,18,19, 11/1e, 12/21, 15/8,16,-18, 16/15, 18/15,20,8, 19/8,2a, 20/18, 21/12e; HR MALDI MS (positive ions) *m/z* 1073.5178 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{23}$-Na, 1073.5146), 1089.49 $[M + K]^+$.

Mycaloside D (4): colorless solid, mp 209−213 °C; $[\alpha]^{25}_D$ −32.2° (*c* 0.6, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 3/4,1a,1-6-OAcGlc, 4/3,6,16-OAcGlc, 6/7,4, 7/6, 8/15,18,19, 11/1e, 12e/21, 15/8,16,-18, 16/15, 18/15,20,8, 19/8, 20/18, 21/12e; HR MALDI MS (positive ions) *m/z* 1129.5462 $[M + Na]^+$ (calcd for $C_{53}H_{86}O_{24}$-Na, 1129.5408), 1145.52 $[M + K]^+$.

Mycaloside E (5): colorless solid, mp 221−225 °C; $[\alpha]^{25}_D$ −25.5° (*c* 0.45, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1a/3, 3/1a,1-Glc, 4/1-Glc, 6/7, 7/6, 8/15,18, 12/21, 15/8,16, 16/15, 18/20,8, 20/18, 21/12e; HR MALDI MS (positive ions) *m/z* 1057.5148 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{22}Na$, 1057.5196), 1073.48 $[M + K]^+$.

Mycaloside F (6): colorless solid, mp 213−216 °C; $[\alpha]^{25}_D$ −38.0° (*c* 0.38, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 2a/19, 3/4,1a,1−6-OAcGlc, 4/3,6,1-6-OAcGlc, 6/7,4, 7/6, 8/18,19, 9/14, 11/1e, 14/9, 16/22,23, 18/20,8, 19/8,2a, 20/18; HR MALDI MS (positive ions) *m/z* 1115.5228 $[M + Na]^+$ (calcd for $C_{52}H_{84}O_{24}Na$, 1115.5251), 1131.46 $[M + K]^+$.

Mycaloside G (7): colorless solid, mp 210−214 °C; $[\alpha]^{25}_D$ −27.2° (*c* 0.68, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 2a/19, 3/4,1a,1-Glc, 4/3,6,1-Glc, 6/7,4, 7/6, 8/18,19, 9/14, 11/1e, 14/9, 16/22,23, 18/20,8, 19/8,2a, 20/18; HR MALDI MS (positive ions) *m/z* 1073.5084 $[M + Na]^+$ (calcd for $C_{50}H_{82}O_{23}Na$, 1073.5146), 1089.47 $[M + K]^+$.

Mycaloside H (8): colorless solid, mp 205−209 °C; $[\alpha]^{25}_D$ −31.6° (*c* 0.3, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1e/11, 1a/3, 3/4,1a,1-Glc, 4/3,6,1-Glc, 6/7,4, 7/6, 8/18,19, 9/14, 11/1e, 12e/21, 14/9, 18/20,8, 19/8, 20/18, 21/12e, 26/28, 27/28, 28/26,27; HR MALDI MS (positive ions) *m/z* 1085.5187 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{23}Na$, 1085.5146), 1101.50 $[M + K]^+$.

Mycaloside I (9): colorless solid, mp 222−225 °C; $[\alpha]^{25}_D$ −6.5° (*c* 0.7, MeOH); ^{1}H and ^{13}C NMR spectra, see Tables 1−3; NOESY correlations (H/H) 1a/3, 3/5,1a,1-Glc, 5/3, 6/7, 7/6,15, 9/14, 12e/21, 14/9, 15/7,16, 16/15, 18/20, 20/18, 21/12e, 25/28, 26/28, 27/28, 28/26,27,25; HR MALDI MS (positive ions) *m/z* 1085.5481 $[M + Na]^+$ (calcd for $C_{52}H_{86}O_{22}Na$, 1085.5509), 1101.52 $[M + K]^+$.

Isolation and Analysis of the Free Sterol Fraction. An ethanolic extract of *M. laxissima* was concentrated in vacuo and separated by column chromatography on a Sephadex LH-20 in the system EtOH−$CHCl_3$, 1:1. Part of the nonpolar fraction (0.73 g) was purified by column chromatography on Si gel KSK (Russia), using the solvent system hexane−ethyl acetate, 5:1, followed by HPLC on an Ultrasphere-Si (250 × 10 mm) column in the solvent system hexane−ethyl acetate, 5:1, to obtain a free sterol fraction (5.9 mg). The analysis of this fraction was carried out by GLC and GLC-MS methods using cholesterol and clionasterol as standards.

Sea Urchin *S. nudus* Assay. The sea urchin *Strongylocentrotus nudus* sperm, eggs, and embryos were used as test material for bioassays according to the method of Kobayashi.[31] Glycosides were dissolved in DMSO. The final concentration of DMSO in the test solution during the experiments did not exceed 1%. All experiments were repeated in triplicate. The results were expressed as a percentage relative to the controls and plotted. Means and standard errors for each treatment were calculated, and EC_{50} values were estimated using SigmaPlot 3.02 software (Jandel Corporation).

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References and Notes

(1) Minale, L.; Riccio, R.; Zollo, F. In *Progress in the Chemistry of Organic Natural Products*; Herz, H., Kirby, G. W., Moore, R. E., Steglich W., Tamm, Ch., Eds.; Springer-Verlag: New York, 1993; Vol. 62, pp 75−308.
(2) Iorizzi, M.; De Marino, S.; Zollo, F. *Curr. Org. Chem.* **2001**, *5*, 951−973.
(3) Stonik, V. A. *Russ. Chem. Rev.* **2001**, *70*, 673−715.
(4) Kitagawa, I.; Kobayashi, M.; Okamoto, Y.; Yoshikawa, M.; Hamamoto, Y. *Chem. Pharm. Bull.* **1987**, *35*, 5036−5039.
(5) Schmitz, F. J.; Ksebati, M. B.; Gunasekera, S. P.; Agarwal, S. *J. Org. Chem.* **1988**, *53*, 5941−5947.
(6) Kobayashi, M.; Okamoto, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1991**, *39*, 2867−2877.
(7) Espada, A.; Jimenez, C.; Rodriguez, J.; Crews, P.; Riguera, R. *Tetrahedron* **1992**, *48*, 8685−8696.
(8) Lee, H.-S.; Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 915−919.
(9) Carmely, S.; Roll, M.; Loya, Y.; Kashman, Y. *J. Nat. Prod.* **1989**, *52*, 167−170.
(10) D'Auria, M. V.; Gomez Paloma, L.; Minale, L.; Riccio, R. *Tetrahedron* **1992**, *48*, 491−498.
(11) Gulavita, N. K.; Wright, A. E.; Kelly-Borges, M.; Longley, R.; Yarwood, D.; Sills, M. A. *Tetrahedron Lett.* **1994**, *35*, 4299−4302.
(12) Jaspars, M.; Crews, P. *Tetrahedron Lett.* **1994**, *35*, 7501−7504.
(13) Antonov, A. S.; Kalinovsky, A. I.; Stonik, V. A.; Evtuschenko, E. V.; Elyakov, G. B. *Russ. Chem. Bull.* **1994**, *43*, 1265−1269.
(14) Antonov, A. S.; Kalinovsky, A. I.; Stonik, V. A. *Tetrahedron Lett.* **1998**, *39*, 3807−3808.
(15) Antonov, A. S.; Kalinovsky, A. I.; Dmitrenok, P. S.; Stonik, V. A. *Bioorg. Khim.* **2002**, *28*, 209−214.
(16) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O. *Eur. J. Org. Chem.* **1999**, *2*, 231−238.
(17) Camagnuolo, C.; Fattorusso, E.; Tadlialatela-Scafati, O. *Tetrahedron* **2001**, *57*, 4049−4055.
(18) Hirota, H.; Takayama, S.; Miyashiro, S.; Ozaki, Y.; Ikegami, S. *Tetrahedron Lett.* **1990**, *31*, 3321−3324.
(19) Ryu, G.; Choi, B. W.; Lee, B. H.; Hwang, K. H.; Lee, U. C.; Jeong, D. S.; Lee, N. H. *Tetrahedron* **1999**, *55*, 13171−13178.
(20) Kalinovsky, A. I.; Antonov, A. S.; Afiyatullov, Sh. Sh.; Dmitrenok, P. S.; Evtuschenko, E. V.; Stonik, V. A. *Tetrahedron Lett.* **2002**, *43*, 523−525.
(21) Wright, J. L. C.; McInnes, A. G.; Shimizu, S. S.; Smith, D. G.; Walter, J. A.; Idler, D.; Khalil, W. *Can. J. Chem.* **1978**, *56*, 1898−1903.
(22) Wilson, W. K.; Sumper, R. M.; Warren, J. J.; Rogers, P. S.; Ruan, B.; Schroepfer, G. J. *J. Lipid Res.* **1996**, *37*, 1529−1555.
(23) D'Auria, M. V.; Riccio, R.; Uriarte, E.; Minale, L.; Tanaka, J.; Higa, T. *J. Org. Chem.* **1989**, *54*, 234−239.
(24) Allinger, N. L.; Hermann, R. B.; Djerassi, C. *J. Org. Chem.* **1960** *25*, 922−927.
(25) Burgoyone, D. L.; Andersen, R. J.; Allen, T. M. *J. Org. Chem.* **1992**, *57*, 525−528.
(26) Shoji, N.; Umeyama, A.; Shin, K.; Takeda, K.; Arihara, S.; Kobayashi, J.; Takei, M. *J. Org. Chem.* **1992**, *57*, 2996−2997.
(27) Kobayashi, J.; Shinonaga, H.; Shigemori, H.; Umeyama, A.; Shoji, N.; Arihara, S. *J. Nat. Prod.* **1995**, *58*, 312−318.
(28) Sperry, S.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 29−32.
(29) Keyzers, R. A.; Northcote, P. T.; Webb, W. *J. Nat. Prod.* **2002**, *65*, 598−600.
(30) Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly, M. *J. Org. Chem.* **1999**, *64*, 6706−6709.
(31) Kobayashi, N. In *Ecotoxicological Testing for the Marine Environment;* Persoone, G., Jaspers, E., Claus, C., Eds.; State University of Ghent and Institute of Marine Scientist Research: Bredene: Belgium, 1984; Vol. 1, pp 798−800.

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